CirmBlots

A Technologies Advancement Company

Multiple Tissues Northern Blots
User Manual
UM-1008-0
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For Research Use Only

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I. Introduction

CirmBlots' Blots are ready-to-hybridize Multiple Tissue Northern Blot optimized for intact mRNA and maximum sensitivity. CirmBlots' Blots can be used for the relative quantitative or/and specific messages, to assess gene expression across all tissues. Identifying alternative spliced transcripts and to determine the size of specific messages. CirmBlots' premium quality pre-made blots allow the user to precede a rapid gene expression analysis without the time consuming inconvenience of RNA isolation, gel preparation, and transfer procedures.

CirmBlots' Multiple Tissue Northern Blots contain 2-5 ug of poly A+ RNA per lane. The RNAs are carefully isolated from various human and mouse tissues. CirmBlots' Blots are prepared by transferring gel fractionated mRNA to a positively-charged nylon membrane, which ensures the complete transfer of even long messages. We assured the transfer of the largest transcript (9 Kb). CirmBlots' blots provide greater sensitivity than ordinary Northern Blots, CirmBlots' Blots RNA are concentrated to a small area by using specially designed combs. A lane of marker, which sizes are 281, 725, 955, 1383, 1908, 2604, 4981 and 6583 bases is included on each CirmBlots' blot.

CirmBlots Northern Blots give maximum sensitivity and utility. They can be stripped and re-probed several times. Most of the CirmBlots contain 2-5 ug of poly A+ RNA from human or mouse tissue. CirmBlots' Blots have been clipped in the top left-hand corner to provide an orientation for the markers and RNAs. Note diagram on the following page for RNAs orientation.

To obtain more CirmBlots Blots information and CirmBlots publications please Visit: www.cirmblots.com

II. List of Components

Stored **unused** CirmBlots' Blots at room temperature in a sealed plastic bag away from light.

Store **Used** CirmBlots' Blots at 4° C in a sealed plastic bag until needed.

Store control probe at -20° C.

- 1 **CirmBlots' Northern Blot, Premium Quality RNA**
- 20 ml **Hybridization Solution**

III. Special Handling of RNA is required, Lab. Ware and solutions

Special Handling of RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not required cafactars to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plastic ware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds, and are the most common sources of RNase contamination. Always wear a latex or vinyl gloves while handling RNA samples and reagents to prevent RNase contamination from the surface of the skin and from dusty laboratory equipment. Change gloves frequently and keep laboratory equipment, tubes etc. covered and closed as much as possible at all time.

Lab ware-Laboratory ware:

- **Disposable plasticware**

  The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

- **Non-Disposable plasticware**

  Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1mM EDTA followed by RNase free water. Alternatively chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

- **Glassware:**

  Glassware should be treated before use, to ensure that it is RNase-free. Glassware used for RNA work should be cleaned well with detergent, thoroughly rinsed, and oven baked at 240 ^{o}C for four or more hours (overnight, if convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethylpyrocarbonate). Fill Glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37 ^{o}C, then autoclave or heat to 100 ^{o}C for 15 minutes to eliminate residual DEPC.

  Note: Corex tubes should be rendered RNase-free by treatment with DEPC and not by baking since baking increases the failure rate of the type of the tube during centrifugation.

- **Electrophoresis tanks:**

  Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.

- **Solutions:**

  Solutions (water and other solutions) should be treated with 0.1% DEPC*****. DEPC is a strong but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37 ^{o}C. Autoclave for 15 minutes to remove any trace of DEPC, DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carboxymethylation. Carboxymethylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100 ^{o}C for 15 minutes.

  Note: Oligotex buffers are guaranteed RNase-free without using DEPC treatment and are free of

any DEPC contamination.

***** DEPC is a suspected carcinogen and should be handled with great care. Wear Goggles and gloves and use a fume hood when using this chemical.

****** Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check with the supplier's instructions.

Preparation of additional solutions please follows the next section.

IV. Preparation of Additional Solutions Required

- **DEPC Water**

- **50 mM NaOH-10 mM NaCl**

- **0.1 Tris-Cl (pH 7.4)**

- **20X SSC**

  3 M NaCl

  0.3 M Sodium Citrate (pH 7.0)

  <u>To Prepare 20X SSC Solution</u>:

  1. Dissolve 173.3 g NaCl and 88.2 g of Sodium Citrates in 800 ml of DEPC water.
  2. Adjust the pH to 7.0 with a few drops of the 10NaOH solution of the concentrated Acetic Acid.
  3. Adjust the volume to 1 liter
  4. Auto clave

- **Wash Solution 1**

  2X SSC

  0.05% SDS

- **Wash Solution 2**

  0.1X SSC

  0.1% SDS

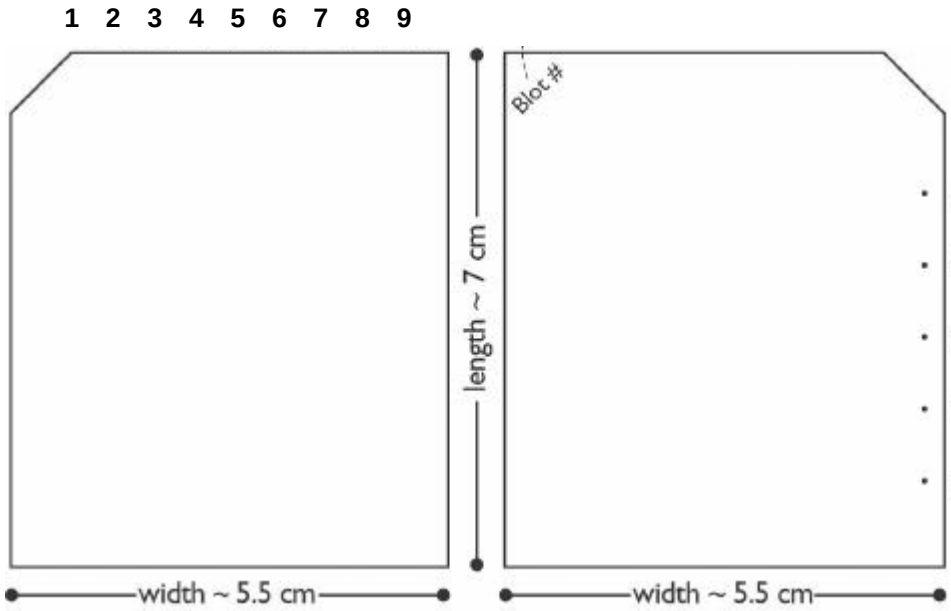
V. CirmBlots Blots Orientation; Lanes Identification

Lanes are in the following orientation order left to right:

e.g. CirmBlots item HB02:

(1) Markers (2) Heart (3) Brain (4) Placenta (5) Lung (6) Liver (7) Skeletal Muscle (8) Kidney (9) Pancreas

Sample RNA Lanes **Blot Numbering**

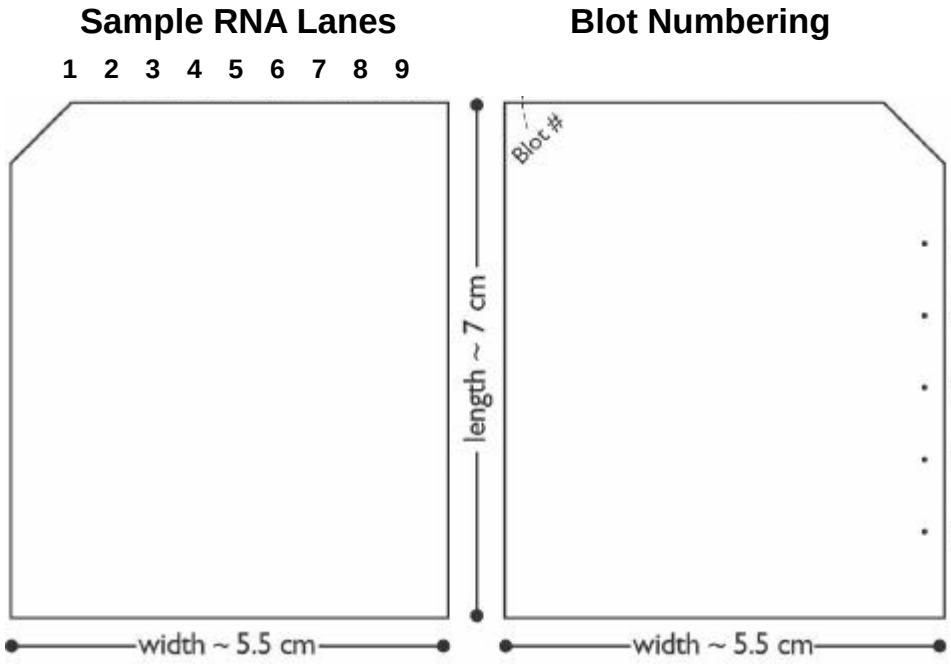


All CirmBlots products are sold for intended for research use only unless otherwise indicated. CirmBlots products are not intended for diagnostic or drug purposes

VI. Hybridization of CirmBlots Blots

A. To Hybridize CirmBlots:

1. Remove the CirmBlots from the sealed plastic bag.
2. Add sufficient pre-warmed Hybridization Solution to keep the blot uniformly wet, and Prehybridize at the desired hybridization temperature.
3. Add probe to the hybridization solution, hybridize the CirmBlots Blots at the desired hybridization temperature. Generally, we recommend the use of 1 x 10^6 cpm/ml of DNA or RNA probe, 1-10 pM nonisotopic DNA probe or 0.1 nM nonisotopic RNA probe. The hybridization time is typically overnight, depending on the type of hybridization buffer used, and the abundance of the target and type of probe used (RNA or DNA)
4. Perform the post-hybridization washes. Generally the post-hybridization washes are performed at the hybridization temperature for times empirically determined to give optimal specific signal to background signal ratios. We recommend two low stringency washes of 15 minutes each and two high stringency washes of 15 minutes each.
5. Expose the blot to X-ray film (^{32}P probe) in a cassette. For nonisotopic detection, follow the kit or reagent manufacture's directions.
6. The following diagrams indicated CirmBlots RNA and Lanes locations:


Sample RNA Lanes
Blot Numbering
1 2 3 4 5 6 7 8 9
Blot #
length ~ 7 cm
width ~ 5.5 cm
width ~ 5.5 cm
Front of Northern Blot
(Nucleic acid on the side)
Back of Northern Blot
(Nucleic acid on other side)

VII. Protocol

I. Hybridization

- Prehybridization membrane for 1-4 hours at 42 ^{o}C with 5-10 ml Prehybridization buffer.
- Heat labeled probe for 3 minutes at 95 ^{o}C, cool on ice
- Discard Prehybridization buffer, add hybridization buffer and probe, incubate at 42 ^{o}C at least 8 hours.
- Wash membrane 1 x 15 minutes with 2 x SSC at room temperature.
- Wash membrane 2 x 15 minutes with 2 x SSC with 0.1% SDS at 65 ^{o}C.
- Wash membrane 1 x 15 minutes with 0.1 x SSC with 0.1% SDS at 65 ^{o}C (optional).
- Wrap the membrane with saran wrap and expose film at -80 ^{o}C overnight.

Note: Never let the membrane dry until the blot is stripped.

If you are using hybridization bottles, make sure that the marked side of the membrane is flush against the side of the bottle. Bubbles between the membrane and the bottle can prevent hybridization to those areas.

II. Stripping and Rehybridization

- Wash membrane for 0.5-3 hours in strip solution at 70 - 80^{o}C until on radioactivity can be detected on the membrane.
- The membrane can now be air dried and stored at room temperature
- For rehybridization several times following the hybridization protocol.

VIII. Troubleshooting Guide

A. High background

Lengthening the time of low or high stringency washes rarely decreases high background signals. Remove the probe from the blot as you would for reprobing. Monitor the process with a Geiger counter to verify cpm is reduced to almost background levels. If there is still a high background, store the blot covered in plastic wrap at -20° C for 2-3 half lives. The half life of ^{32}P is 14 days.

High background can occur for several reasons:

- Unincorporated [^{32}P] dNTPs are not fully removed from the probe. Check the ratio of TCA-precipitable cpm to total cpm in the final probe preparation. Adjust chromatography conditions to achieve a ratio of >95%. We also recommend purifying your hybridization probe by exclusion chromatography.
- Average size of the DNA probe is too large. Optimal sizes range from 200-800 nucleotides. Increase the ratio of random primers to template DNA
- Concentration of probe in hybridization solution is too high. For DNA probes, do not exceed 2 x 10^6 cpm/ml. For oligonucleotide probes, do not exceed 5 x 10^6 cpm/ml.

B. Hybridization signals absent or very weak

If signals are not generated after 1-2 days of x-ray film exposure, the hybridization probe may have a low specific activity. Make a new probe with fresh ^{32}P.

The specific activity of your probe should always be >5 x 10^8 cpm/µg, and, for maximum sensitivity >1 x 10^9 cpm/µg. If specific activity is still low upon repeating the labeling, you may be using too little DNA. We use 25-50 ng measured from the OD_{260} of a concentrated stock. If you have a small quantity of DNA, electrophorese the amount you have been using on an agarose gel, next to a known amount of DNA markers and estimate the amount of probe DNA. If a clear ethidium bromide-stained band is not observed, you have less than 25-50 mg, and therefore, should use 2-3 times more DNA for probe labeling. If this fails, optimize labeling conditions with a known amount of control probe such as β-actin.

C. Probe not fully homologous to target

If you are using a cross-species probe, you may need to reduce the stringency of your final wash by using Wash Solution 1 instead of Wash Solution 2 and decreasing the temperature by 5-10° C. When using a synthetic oligonucleotide probe, ensure that the probe is completely homologous to the target.

D. Inability to strip and reprobe

If you are unable to reprobe the blot, the membrane may not have been stripped completely or may have been allowed to dry or partially dry. If a membrane is allowed to even partially dry, subsequent removal of the probe may be impossible. To prevent drying after your final wash, shake off excess solution with forceps (do not blot-dry) and wrap the blot immediately with plastic wrap. When reprobing, uncover the blot, immediately place it in heated sterile water containing 0.5% SDS, and follow the rest of the protocol provided for removing probes. If the membrane has not partially dried, see Section A above.

E. Signal Decreases after two reprobings

This problem is especially common for low abundance genes. You may not see signals as strong as those observed for high-abundance transcripts such as β-actin.